Supplementary Information

Targeted Codelivery of Prodigiosin and Simvastatin Using Smart BioMOF:

Functionalization by Recombinant Anti-VEGFR1 scFv

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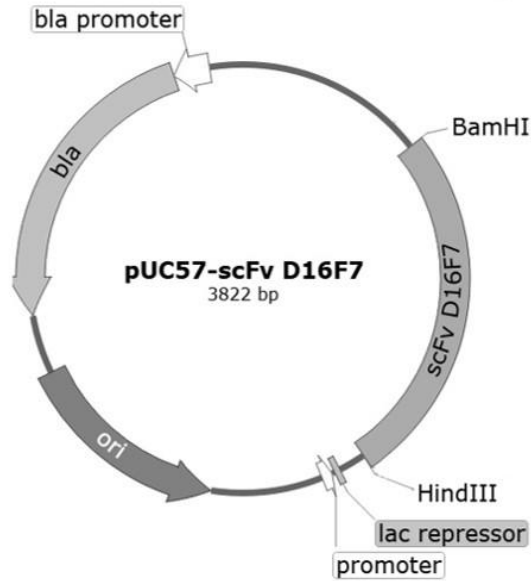Hamid Reza Karbalaei-Heidari, karbalaei@shirazu.ac.ir; Hamid.karbalaeiheidari@umanitoba.ca

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Table S1. Summary list of IC$_{50}$ values for various formulations after 24 and 48 h incubation on different cell lines. * Indicates amount of drug loaded onto the BioMOFs.
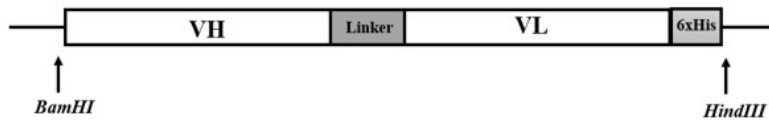
Cell line	Incubation time	IC50								
		PG (µg/ml)	PG-NP (µg/ml)*	PG-NP-D16F7 (µg/ml)*	SIM (µg/ml)	SIM-NP (µg/ml)*	SIM-NP-D16F7 (µg/ml)*	PG-SIM (µg/ml)	PG-SIM-NP (µg/ml)*	PG-SIM-NP-D16F7 (µg/ml)*
MCF-7	24 h	0.73±0.113	2.58±0.262	1.85±0.504	9.22±0.321	5.85±0.5	4.6±0.723	5.99±0.29	3.70±1.96	2.18±0.51
	48 h	0.316±0.038	1.62±0.403	1.59±0.462	5.11±0.622	4.48±0.628	3.1±0.5	3.11±0.293	2.46±0.47	1.49±0.3
LnCap	24 h	0.950±0.118	2.68±0.650	2.54±0.211	9.63±0.497	5.36±0.461	3.86±0.254	6.58±1.4	4.29±0.424	3.83±1.21
	48 h	0.45±0.069	2.26±0.410	1.57±0.686	7.64±0.492	4.03±0.374	2.84±0.24	2.99±0.36	2.88±0.282	2.29±0.148
U87MG	24 h	0.97±0.078	3.13±0.294	1.17±0.421	8.13±0.805	5.94±1.1	3.86±0.14	4±0.794	3.17±0.202	2.514±0.258
	48 h	0.21±0.055	1.83±0.04	0.91±0.34	6.01±0.141	4.02±0.97	2.31±0.24	0.61±0.733	2.31±0.463	1.80±0.105
HSF	24 h	1.85±0.163	3.64±0.7	2.88±0.11	14.56±0.57	7.48±1.51	5.34±0.866	7.10±0.424	6.89±1.24	5.43±1.37
	48 h	0.63±0.003	2.59±0.091	1.65±0.4	9.12±0.67	5.59±0.82	3.87±1.02	3.84±0.212	3.43±0.640	2.49±0.66

Figure. S1. Schematic representation of the construct and expression vectors used in this study. A) Structure of pUC-57- D16F7 scFv which was synthesized by Genscript. B) Design of the D16F7 scFv gene sequence, consisting of VH and VL nucleotide sequences joined by a flexible peptide linker sequence [(G4S)3] and a C-terminal 6 × His tag. **C).** Schematic map of the pMal-c2X-D16F7 scFv construct used to express MBP-D16F7 scFv in *E. coli*. D) Nucleotide sequence of the variable heavy (VH) domain, flexible linker, and variable light (VL) domain of D16F7 scFv.

A)

B)

C)

D)

Variable Heavy (VH) Domain:

GATGTGCTGATGACTCAGACCCCTCTGTCTTTGCCGGTGAGCCTGGGCGATCAGGCG
TCTATTAGTTGCCGCTCTAGCCAGAGCATTGTGCATTCCAACGGCAACACCTATCTG
GAATGGTTTTTGCAGAAGCCAGGCCAAAGCCCGAAACTTCTGATCTATAAAGTGAG
CAACCGCTTTAGCGGTATTCCGGATCGCTTTAGCGGCAGCGGTAGCGGCACCGATTT
TACCCTGAAAATTTCTCGTGTGGAGGCGGAAGACCTGGGTGTTTATTTTTGCTTTCAG
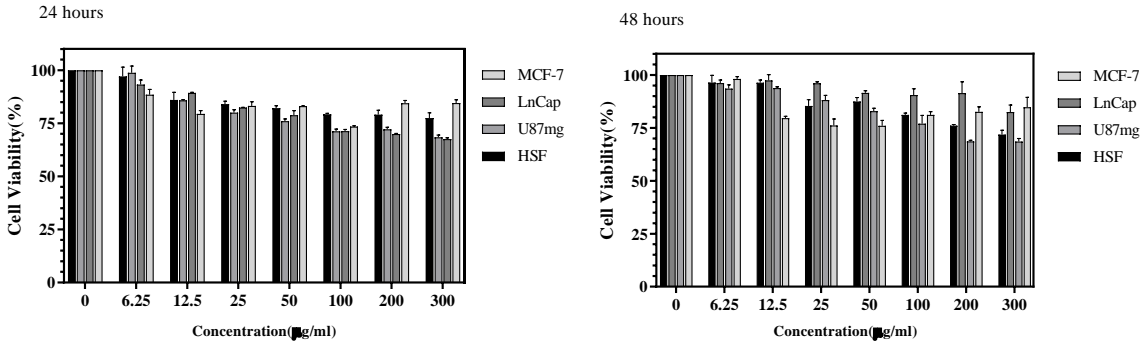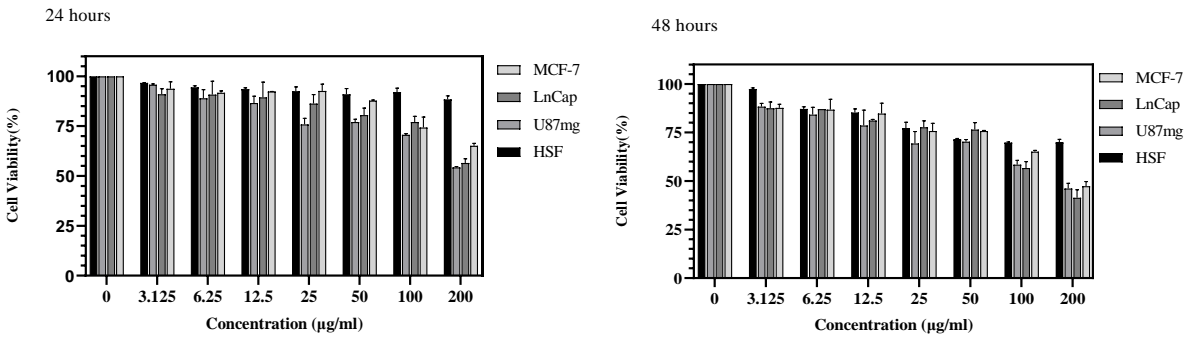GGCAGCCATGTGCCGTATACCTTTGGTGGCGGCACCAAACTGGAAATTAAA

Nucleotide Sequence of Linker:

GGCGGTGGCGGTAGCGGTGGTGGCGGTAGCGGTGGCGGCGGTAGC

Variable Light (VL) Domain:

GATGTGCTGATGACTCAGACCCCTCTGTCTTTGCCGGTGAGCCTGGGCGATCAGGCG
TCTATTAGTTGCCGCTCTAGCCAGAGCATTGTGCATTCCAACGGCAACACCTATCTG
GAATGGTTTTTGCAGAAGCCAGGCCAAAGCCCGAAACTTCTGATCTATAAAGTGAG
CAACCGCTTTAGCGGTATTCCGGATCGCTTTAGCGGCAGCGGTAGCGGCACCGATTT
TACCCTGAAAATTTCTCGTGTGGAGGCGGAAGACCTGGGTGTTTATTTTTGCTTTCAG
GGCAGCCATGTGCCGTATACCTTTGGTGGCGGCACCAAACTGGAAATTAAA

Figure. S2. Cytotoxicity effect of A) the blank BioMOF, B) the free MBP-D16F7 scFv, and C) the unloaded Ca-Gly-D16F7 after 24 and 48 h incubation on the cancer cells (U87mg, LnCap, MCF-7) and normal cell (HSF). Data represent mean ± SD.
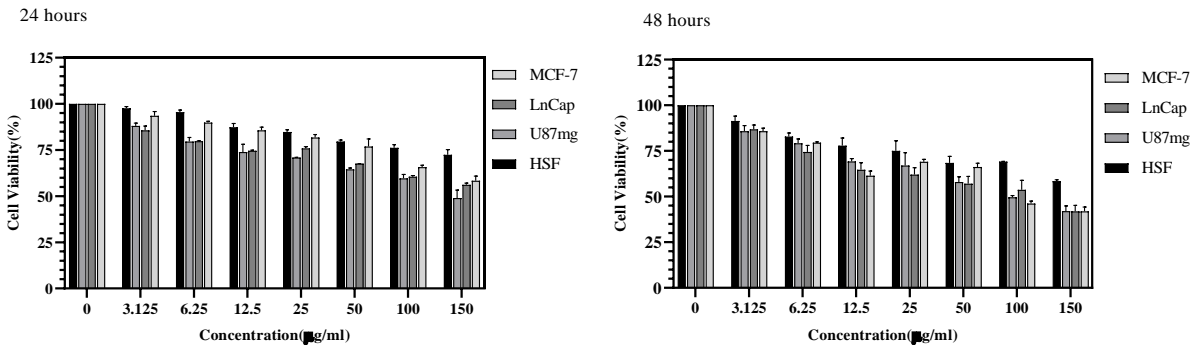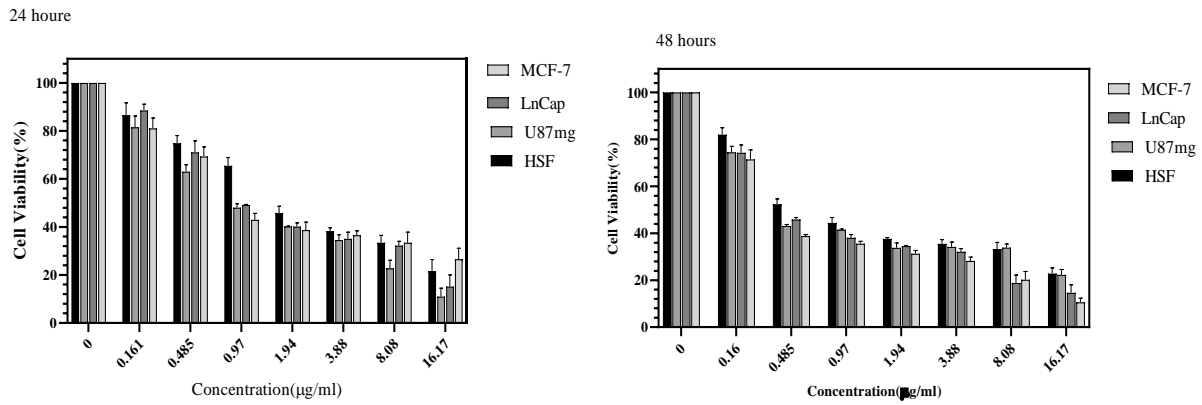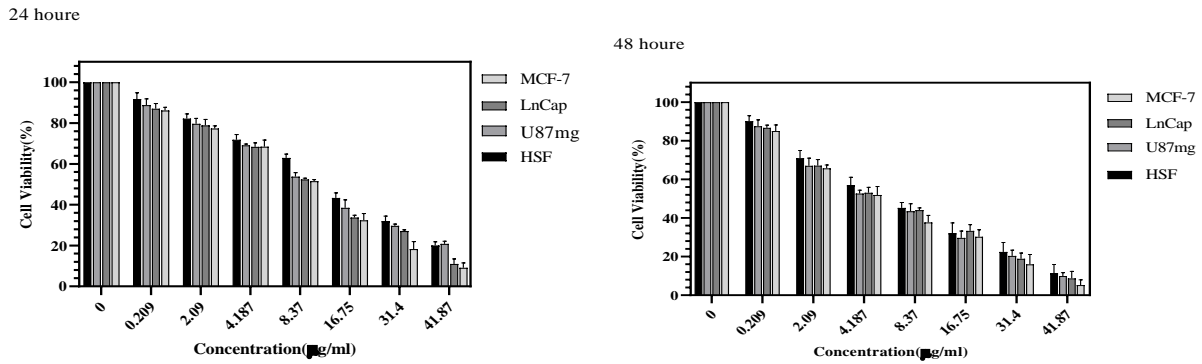
A)

B)

C)

Figure. S3. Percentage of cell viability of various cell lines after 24 h and 48 h incubation by A) the PG, B) the SIM, C) the PG-NP, D) the PG-NP-D16F7, E) the SIM-NP, F) the SIM-NP-D16F7, G) the PG+SIM-NP, and H) the PG+SIM-NP-D16F7 on various cell lines after 24 h and 48 h incubation. The data are mean values ± SD.
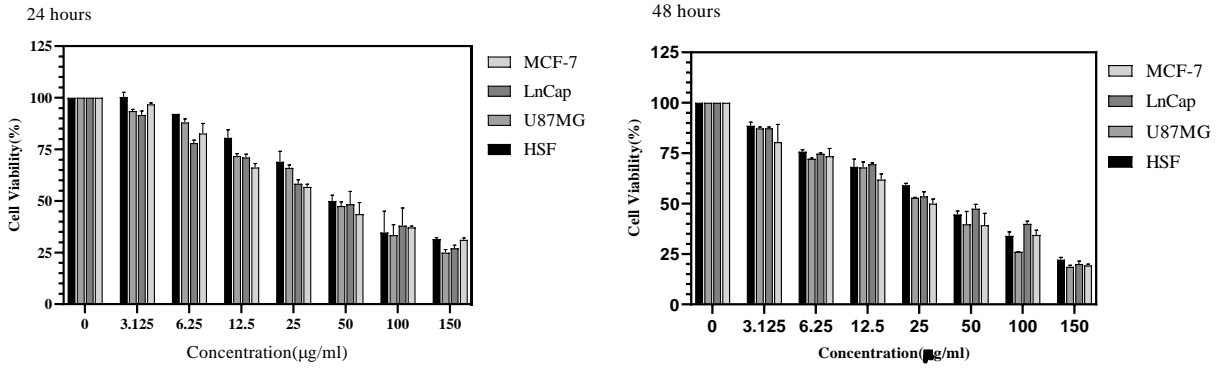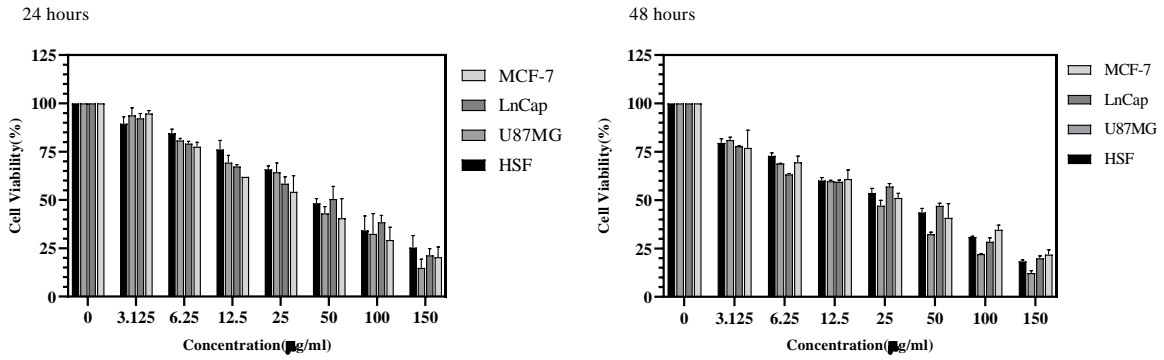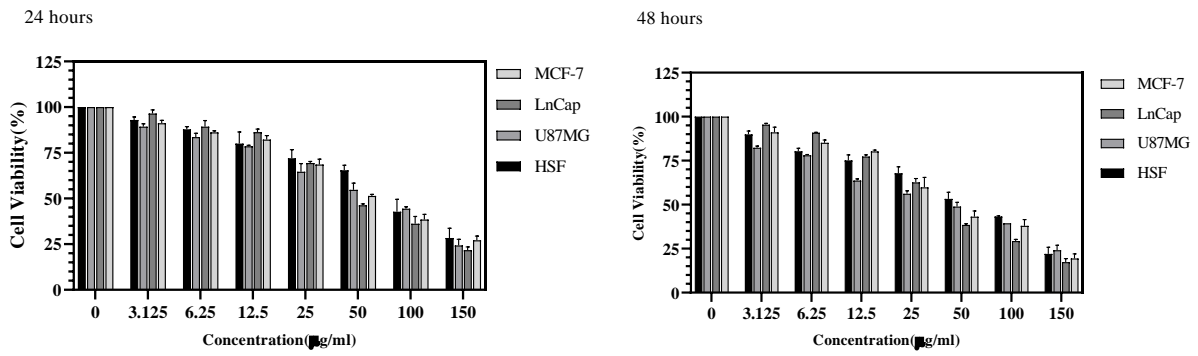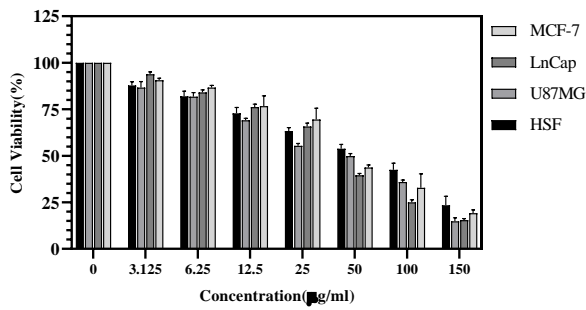
A)

24 houre

48 hours
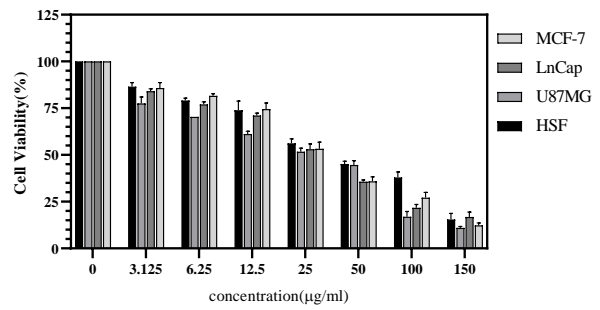

B)
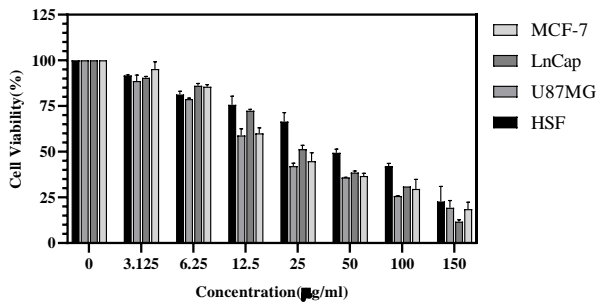
24 houre

48 houre
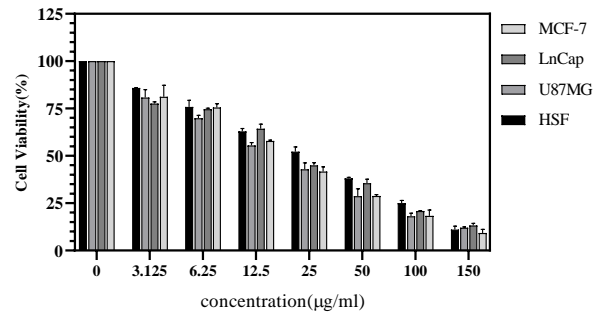
C)

D)

E)

F)

24 hours

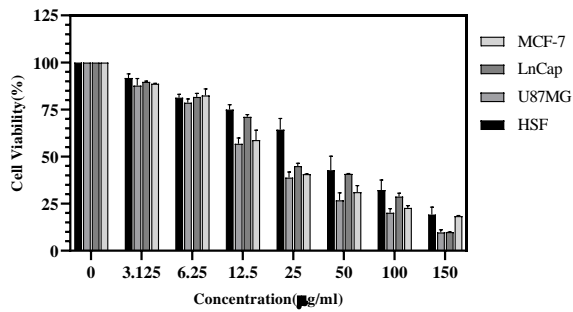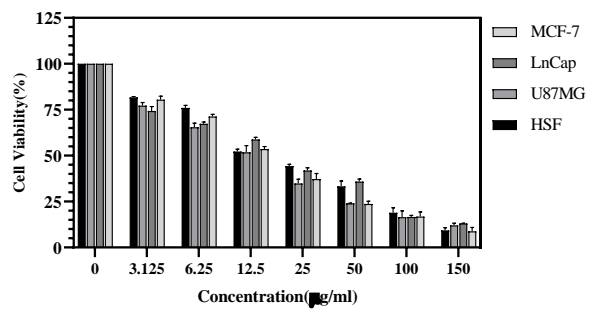48 hours

G)

24 hours

48 hours

H)

24 hours

48 hours

Figure. S4. The combination index (CI) plot of PG+SIM and PG+SIM-NP after 24 and 48 h incubation on A) MCF-7, B) LnCap, C) U87MG, and D) HSF cell lines. Data represent the mean ± SD.
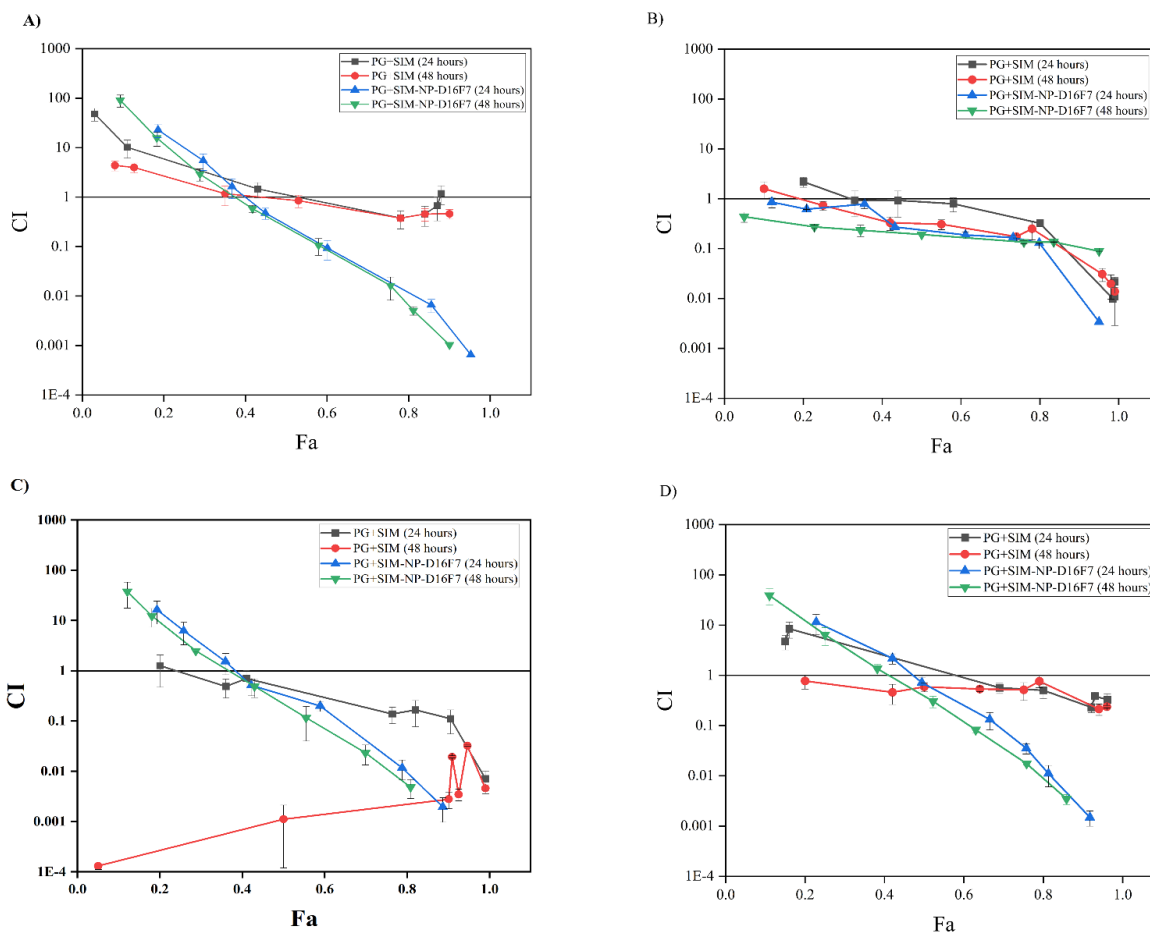
Figure. S5. Cellular uptake monitoring of PG+SIM, the PG+SIM-NP and PG+SIM-NP-D16F7 after addition to cell lines on A) MCF-7, B) LnCap, C) U87MG, and HSF. Images were taken after 30 min, 90 min, and 24 h of incubation.

C)

	PG-SIM			PG-SIM-NP			PG+SIM-NP-D16F7		
	PG	DAPI	Merged	PG	DAPI	Merged	PG	DAPI	Merged
30 min									
90 min									
24 h									

D)

	PG-SIM			PG-SIM-NP			PG+SIM-NP-D16F7		
	PG	DAPI	Merged	PG	DAPI	Merged	PG	DAPI	Merged
30 min									
90 min									
24 h									